

Scotland's Rural College

Feed-restricted broiler breeders: state-dependent learning as a novel welfare assessment tool to evaluate their hunger state?

Buckley, LA; Sandilands, V; Hocking, PM; Tolkamp, BJ; D'Eath, RB

Published in:
Applied Animal Behaviour Science

DOI:
[10.1016/j.applanim.2015.01.006](https://doi.org/10.1016/j.applanim.2015.01.006)

Print publication: 01/01/2015

Document Version
Peer reviewed version

[Link to publication](#)

Citation for pulished version (APA):

Buckley, LA., Sandilands, V., Hocking, PM., Tolkamp, BJ., & D'Eath, RB. (2015). Feed-restricted broiler breeders: state-dependent learning as a novel welfare assessment tool to evaluate their hunger state? *Applied Animal Behaviour Science*, 165, 124 - 132. <https://doi.org/10.1016/j.applanim.2015.01.006>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Feed restricted broiler breeders: State-dependent learning as a novel welfare assessment tool to evaluate their hunger state?

Buckley, Louise A.^{1±}, Sandilands, Victoria.¹, Hocking, Paul M. ², Tolkamp, Bert J.¹ & D'Eath, Rick B.¹

¹SRUC, West Mains Road, Edinburgh, EH9 3JG, UK.

²The Roslin Institute and Royal (Dick) School of Veterinary Studies, The Roslin Institute Building, Easter Bush, Midlothian, EH26 9RG, UK

± Corresponding author:

Louise A. Buckley,

Department of Animal Production, Welfare and Veterinary Sciences,

Harper Adams University,

Newport,

Shropshire,

TF10 8NB

UK

Tel: +44 (0) 1952 815147

Fax: Not available

Email: lbuckley@harper-adams.ac.uk

Abstract

This paper reports three experiments that aimed to validate the use of state – dependent learning (SDL) as a novel welfare assessment tool to evaluate the hunger state of feed restricted broiler breeders.

In each experiment, birds alternated every two days between two food rations: quantitative feed restriction (QFR) and *ad libitum* access to the same feed (AL). Each food ration was paired with a different, end of day, coloured food reward. It was predicted that the reward associated with hunger (QFR FR) would be preferred to the food reward associated with AL (AL FR) in a subsequent choice test. The SDL preference testing took place after 4 and 8 days of training. Each bird was tested twice (once per food ration fed on the test day).

In experiment 1 (pilot, $n = 4$), birds preferred the QFR – associated reward during both tests (mean (\pm S.E.M.) preference: QFR FR: 35.0 (\pm 3.5) g; AL FR: 2 (\pm 1.3) g, but differential food reward intake between hunger states during training confounded the results.

In experiment 2 ($n = 12$) a smaller food reward was used during training to try and equalise intake. The birds preferred the QFR FR in test one only (least significant difference (L.S.D.) = 15.08, $P < 0.05$). The mean (\pm S.E.M.) consumption in test one was: QFR FR: 31.6 (\pm 4.3) g; AL FR: 9.41 (\pm 2.3) g. However, differential reward intake continued to confound the findings.

In experiment 3 ($n = 8$), the food reward was made more palatable by feeding moist and food reward intake during training was equalised between hunger states. During testing, birds continued to show a significant preference in test one only (L.S.D. = 13.73, $P < 0.05$).

It was concluded that SDL – derived preferences observed do exist but are not a robust phenomenon. Therefore, further research is needed to quantify factors influencing SDL development and maintenance before using SDL as a tool to assess hunger in feed restricted broiler breeders.

Keywords:

Broiler breeder; feed restriction; state – dependent learning; animal welfare; choice test; preference test

1. Introduction

State – dependent learning (SDL) is the phenomenon in which an animal shows a preference for something based on the context in which it originally encountered it. An animal that experiences a stimulus linked to a food reward when in a state of high deprivation and another stimulus linked to an identical food reward when in a state of low deprivation will often show a preference for the stimulus associated with a state of high deprivation (Pompilio and Kacelnik, 2005). Furthermore, this preference has been shown to be independent of the animal's current state at the time of the two – way preference testing (e.g. Kacelnick and Marsh, 2002; Kurtz and Jarka, 1968). A state of high deprivation can be induced external to the training situation by food restriction (Vasconcelos and Urcuioli, 2008; Pompilio et al., 2006; Capaldi et al., 1994; Kurtz and Jarka, 1968; Revusky, 1967), by making the animal work hard to access the food reward within the training situation (Gipson et al., 2009; Friedrich and Zentall, 2004; Kacelnick and Marsh, 2002; Clement et al., 2000) or by making the animal wait longer to access the reward (Pompilio and Kacelnik, 2005).

These seemingly irrational preferences are thought to occur because the animal values the same reward differently dependent on its value to the animal at the time that it originally encountered it (Pompilio and Kacelnik, 2005). This may occur due to the increased contrast between hedonic states before and after receiving the food reward in a state of high deprivation during training relative to that experienced when in a state of low deprivation (Clement et al., 2000) and / or due to a perceptual distortion (Pompilio et al., 2006). It has been observed in a wide range of species (fish, Aw et al., 2009; locusts, Pompilio et al., 2006; pigeons, Gipson et al., 2009; Friedrich et al., 2004; rats, Capaldi et al., 1994; Capaldi et al., 1991; Kurtz and Jarka, 1968; and starlings, Pompilio and Kacelnik, 2005; Marsh et al., 2004) indicating that it is a robust phenomenon. This has led researchers to conclude that it must be evolutionarily beneficial or rational in the natural environment (Pompilio et al., 2006) despite this leading to irrational preferences in the laboratory setting.

The phenomenon of preferences caused by SDL has not thus far been applied to animal welfare assessment. It is proposed here to assess the use of SDL as a novel welfare assessment tool to evaluate hunger state in the feed restricted broiler breeder. Quantitative feed restriction is a widely recognised welfare problem for fast – growing broiler breeders (de Jong et al., 2003). Experimentally researchers

have tried to improve welfare by adjusting the quality of the diet by either reducing the energy density, adding appetite suppressants or by a combination of both approaches (e.g. Nielsen et al., 2011; Sandilands et al., 2006, 2005; Hocking et al., 2004; Nielsen et al., 2003; Savory and Lariviere, 2000; Rozenboim et al., 1999). However, whilst these are successful at increasing time taken to consume the ration, it is not clear whether these diets achieve this by improving satiety (a positive affective state) in the broiler breeder (D'Eath et al., 2009). Direct choice test methodologies in which the broiler breeder chooses between either qualitative or quantitative feed restriction have so far not proved successful (Buckley et al., 2011a). Whilst this may be because hungry birds find it more difficult to learn food quality discrimination tasks (Buckley et al., 2011b) it is possible that some other factor affected the lack of preference. Further, choice tests may not actually be measuring preferences determined by altered states of satiety. Thus, there is a need for alternative approaches to identify which, if any, of these alternative diets is more satiating than conventional quantitative restriction. The aim of this series of three related experiments was to identify whether the phenomenon of SDL could be reproduced in broiler breeders that alternated between two feeding levels designed to induce a state of high deprivation (quantitatively feed restricted, QFR) or of low deprivation (fed *ad libitum*, AL). It was hypothesised that there would be an effect of hunger state on bird preference for an end of day, coloured, food reward associated with either high deprivation (very hungry) or low deprivation (close to satiety). It was predicted that the birds would show state dependent learning and learn to prefer the food reward associated with being in a state of high deprivation over one associated with being in a state of low deprivation. Furthermore, it was predicted that this preference would be independent of current state of deprivation (i.e. the preference would be the same, regardless of whether the bird was very hungry or almost satiated at the time of testing). The ultimate purpose was to validate a methodology that could be used as a 'probe of hunger state' to compare the relative states of deprivation induced by QFR and other alternative diets such as qualitative dietary restriction. Data from the pilot study (experiment one) is included as it informs the rest of the study. Differences in housing arrangements between experiments two and three reflect practical facility considerations resulting from a change of research institute.

2.1. Methodology

2.1.1. Subjects

This study used four broilers (as a more readily available model for broiler breeders) aged 28 days. All four birds followed the same dietary treatment and acted as their own control.

Prior to the study the birds had been group reared on a 14:10 h light: dark schedule (day 1 – 28) and spot-brooded (day 1: 31°C, reduced gradually to 21°C on day 21 and then maintained at this temperature thereafter). The birds were fed a commercial starter chick crumb (Farmgate, BOCM Pauls Ltd., Ipswich, Suffolk, UK) *ad libitum* from 1 – 14 days and thereafter feed restricted in line with the recommended daily feed requirements for broiler breeders (Aviagen, 2007). The mean (\pm s.d.) bodyweight of the birds at the beginning of the study at 28 days of age was 551 (\pm 92)g which was approximately 20% heavier than the target bodyweight for broiler breeders at 28 days (440g). They had no previous experimental history.

2.1.2. Housing and husbandry

Each bird was individually housed in a floor pen (1.05m \times 0.45m) with visual access to one other conspecific through a mesh divider. A solid barrier by the feeding area prevented each bird from seeing what food the other bird was eating. Each pen contained wood shavings and a perch. Birds were fed once daily at 09:00h and any food remaining was removed at 16:00h, weighed and the birds' daily feed intake recorded. Water was available *ad libitum*. Birds were maintained on a 9:15h light : dark schedule and a room temperature of 21 - 23°C throughout the study.

Each bird alternated every other day between being fed a quantity of feed equivalent to commercial feed restriction (QFR) and *ad libitum* (AL) access to the same diet between 09:00 – 16:00h. Half the birds started this feeding regime on QFR and half the birds on AL. This schedule was maintained throughout the study, from day 28 until day 65 with the exception of the two days of SDL preference testing and the washout day (described below).

2.1.3. Food and nutrition

The main diet was a custom – made grower mash (Target Feeds, Whitchurch, Shropshire, UK) suitable for broiler breeders from 28 days of age. The diet contained 165 g/kg crude protein and had a metabolisable energy (ME) of 12.1MJ / kg feed.

The food rewards were comprised of the commercial starter chick crumb that the birds were initially reared on (Farmgate, BOCM Pauls Ltd., Ipswich, Suffolk, UK). This diet was used as it had a higher protein and energy density than the grower mash and was expected to be attractive to the birds irrespective of their level of deprivation. The reward diet was either stained red or green using food colouring (Silverspoon, Cambridgeshire, UK). The food rewards were stained by mixing 10 ml of food colouring diluted with 20 ml of water with each 100 g of feed. The feed was then dried to a similar consistency as the original feed by drying in a warm oven (40°C) for approximately 60 minutes.

2.1.4. SDL protocol

2.1.4.1. Training

Training started when the birds were 47 days old. The birds had any feed remaining removed at 16:00h (in practice this was only the birds being fed AL). All birds then received a 15 g food reward that was either stained red or green. Half the birds received the red reward on days when they had been fed QFR and the green reward on days when they had been fed AL (and the remaining birds vice versa). Birds were given 2h to consume the ration. Any ration left was weighed, discarded and each bird's intake recorded.

2.1.4.2. Testing

After eight days of training (i.e. four days per food reward: food ration pairing) birds were tested on day 55 for the presence of a SDL preference. Birds were fed the same food ration that they had received on day 54 on the day of testing. Birds were then given a 'washout' day in which they received no food reward and received the food ration that they did not receive on day 55. Birds were then given another eight days of training (during which they alternated every two days between the two food rations, starting with the diet ration they did not receive on the first day of training) and then tested again on day 65. Thus, each bird was tested twice for the presence of a SDL preference: once on a day when they had been fed QFR and once on a day when they had been fed AL. The order of testing and diet colour associated with QFR was counterbalanced between birds with half tested first on a QFR day and then on an AL day and the other half in the reverse order.

Testing took place within the home pen between 16:00h – 16:30h. Each bird was offered a bowl of red food reward (70 g) and a bowl of green food reward (70 g) and allowed free access to each bowl for three minutes. After three minutes the bowls were removed, the contents weighed and the intake of each was recorded.

2.1.5. Statistical analysis

The sample size was too small for meaningful statistical analysis. Therefore, the results are presented using descriptive statistics only.

2.1.6. Ethical note

This study (and experiment two) were carried out under Home Office license and were approved by SRUC and the Roslin Institute's animal ethics committees. Experiment three was approved by the Harper Adams University College ethics committee and was not carried out under Home Office license but was approved by the local Home Office inspector. Although individually housed, the birds either had visual access to another bird (experiment one and three) or were pair housed overnight (experiment two). The project license did not permit complete social isolation of the birds. The space allowance exceeded the Home Office minimum guidelines. The birds were provided with a perch (experiment one and two) and some wood shavings to facilitate natural behaviours (all experiments). Water was available *ad libitum* for all experiments. The alternating feed schedule ensured that the birds' level of feed restriction was less than under commercial practice and no health problems were expected or observed as a consequence of adding the food colouring. The birds in experiments one and two were euthanased by barbiturate overdose and the birds in experiment three were re – homed at the end of the study.

2.2. Results

2.2.1. Daily food consumption during training and testing

The birds always fully consumed the daily food ration when the ration provided was QFR. The mean (\pm standard error of the mean, S.E.M.) daily intake of QFR over the period of training and testing was 42.6 (\pm 1.2) g. The birds consumed considerably more on days when fed AL and consumed a mean (\pm S.E.M.) daily intake of 114.1 (\pm 12.7) g over the same period.

2.2.2. Food reward consumption during SDL training

The birds always fully consumed the food reward (15 g/day) on days when they were fed the QFR ration. However, the birds failed to fully consume the food reward on the first three days when fed AL. Four birds failed to fully consume the food reward on day one, three birds on day two, and one bird on day three of AL – food reward training. All birds fully consumed the AL food reward on subsequent days. The mean (\pm S.E.M.) individual birds' cumulative daily intake of each food reward by the first SDL test on day nine of training and testing was 60 (\pm 0) g for the QFR-associated food reward and 40.2 (\pm 6.7) g for the AL – associated food reward. The mean (\pm S.E.M.) individual birds' cumulative intake of each food reward (including the food reward consumed during the first SDL test) by the start of the second SDL test was: QFR – associated food reward: 148.4 (\pm 3.0) g; AL – associated food reward: 101.5 (\pm 8.4) g.

2.2.3. SDL preferences

All four birds showed a preference for the food reward associated with high deprivation (i.e. the colour food reward offered on days when the birds were fed QFR). The overall (across both tests) mean (\pm S.E.M.) consumption of the food reward was 2 (\pm 1.3) g for the AL associated food reward and 35 (\pm 3.5) g for the QFR associated food reward. This preference was very similar across both tests (see: figure 1) which indicated that the birds' state of deprivation at the time of testing and the test number did not influence the direction of the preference.

FIGURE 1 GOES HERE

2.2.4. Effect of state on food consumption during each test

The mean (\pm S.E.M.) intake of the birds was 32 (\pm 5.4) g when tested on a day when they were fed AL and 42.1 (\pm 3.9) g when tested on a day when they were fed QFR.

2.2.5. Anecdotal observations

Although this was not formally measured, it was observed that, during testing, consumption of the food reward (both colours) were accompanied by exaggerated gaping of the beak and pronounced 'neck ripples' during swallowing.

2.3. Discussion

Although the sample size was small, the experiment appeared to have validated the use of SDL approaches in hungry broilers, using QFR and AL to induce high and low deprivation states. However, the fact that the birds failed to fully consume the food reward associated with low deprivation (fed AL on that day) during the initial stages of training represented a confounding factor. It could not be certain whether the apparent preference was genuinely due to the birds valuing each food reward differently, dependent on their state of deprivation at the time of encountering the food reward, or due to the differential quantities consumed affecting associative strength or preference in some way. Therefore, although SDL appeared to have potential, further experiments were needed to ensure that all the food reward was consumed under both states of deprivation in order to have confidence in the meaning of any SDL preference observed. Feed intake during the test also appeared to be affected by the state of deprivation at the time of testing. However, the individual bird data suggested that there was overlap between the intake of the birds tested under conditions of high and low deprivation. Therefore, for this parameter is to be assessed appropriately a longer test time was required.

3. SDL Experiment two

3.1. Introduction

The aim of experiment two was to repeat experiment one using a larger sample size and to remove the confounding variable by equalising intake of the food reward during training with each food ration. A longer testing period was also included to try and improve any difference in food reward intake during testing between birds on different diet ration treatments (QFR or AL) on the test days.

3.2. Methodology

3.2.1. Subjects

Twelve Ross 308 broilers (as a model for broiler breeders) were used for this study. These birds had been previously used in a related behaviour study (Buckley et al., 2012) and the housing arrangements and experimental regime are described more fully there. These birds were admitted to this study aged 69 days. Only birds that had been on the QFR/AL feeding regime in this previous study were included

in the methodology/data reported here. The mean (\pm SD) body weight of the birds at the start of this experiment was: 1992 (\pm 149) g.

3.2.2. Housing and husbandry

The birds experienced the same lighting and temperature regime as in experiment one. During the day they were housed in similar pens with the exception that they had no visual access to another bird (due to the constraints of the previous experiment). Instead, they were pair – housed overnight with a bird from the same treatment group. Water was provided *ad libitum* throughout the study; although problems with water delivery during the second SDL training period meant that the birds were occasionally without water for short periods of time (1 – 2 hours day) for approximately three of the training days.

Each bird alternated every other day between QFR and AL (between 09:00 – 15:00h). Any food remaining at 15:00h was removed, weighed and the birds' intake recorded. In practice, this only applied to birds being fed AL. This schedule was maintained throughout the study with the exception of the two days of SDL preference testing and the washout day (described below).

3.2.3. Food and nutrition

The basal diet was identical to that described in experiment one. The red and green food rewards were the same as those described in experiment one.

3.2.4. SDL protocol

3.2.4.1. Training

The training phase was identical to that described in experiment one with the following exceptions. The birds were given 10 g of food reward (instead of 15 g). They were offered this reward at 15:00h and given three hours to consume it.

3.2.4.2. Testing

The testing phase was identical to that described in experiment one with the following exceptions. The birds were given 100 g (instead of 70 g) of each of the different coloured food rewards, the test lasted five minutes per bird (instead of three minutes) and testing took place between 15:00 – 18:00h.

3.2.5. Statistical analysis

All statistical analyses were carried out using Genstat 15th Edition (VSN International, Hemel Hempstead, UK). A repeated measures REML (un-equally spaced time points, unstructured model) was used to assess the following fixed effects: total intake (QFR + AL food reward); diet feed on day of testing; effects of state; colour of the food associated with QFR. The response variate was food reward consumption (g). Bird ID was the random effect. Four time points were created: test one (QFR food reward intake, QFR FR), test one (AL food reward intake, AL FR), test two (QFR FR) and test two (AL FR). Significant differences in consumption of the food rewards associated with QFR or AL were identified post – hoc using least significant differences (L.S.D.) at the 5% level. L.S.D.s were generated by REML. Untransformed data was used for all repeated measures REMLs as the residuals appeared normally distributed.

3.3. Results

3.3.1. Daily food consumption during the period of SDL training and testing

All birds fully consumed the daily allocation of feed when QFR was the offered food ration. The mean (\pm S.E.M.) daily intake on QFR days was: 64.8 (\pm 0.5) g.

As expected, on days when the birds were offered *ad libitum* access they consumed considerably more food. The mean (\pm S.E.M.) daily intake on days when birds were fed AL over the duration of the period of training and testing was 210.6 (\pm 5.6) g.

3.3.2. Food reward consumed on days of SDL training

All birds consumed the full food reward on all days when fed QFR (10 g /day). However, all of the birds failed to fully consume the food reward associated with AL on the first two days of training.

The mean (\pm S.E.M.) cumulative intake (over four days per food reward) by each individual bird of the food reward associated with QFR by the start of the first SDL test was 40 (\pm 0.0) g and for the food reward associated with AL it was 33.8 (\pm 1.7) g. By the start of the second test (eight training days per food reward plus the quantity consumed during the first test) the mean (\pm S.E.M.) cumulative intake (including food reward consumed during the first SDL test was 111.6 (\pm 4.5) g; QFR – associated reward) and 83.2 (\pm 1.4) g; AL – associated food reward).

3.3.3. SDL preference test

The initial analysis indicated that food reward consumption was affected either by test number or what food (QFR or AL) the food rewards were associated with ($F_{3,30} = 3.39$, $P = 0.031$). Post – hoc analysis using least significant differences at the 5% level indicated that this significant effect was due to birds consumed significantly more of the QFR associated food reward than the AL associated food reward during test one (L.S.D = 15.08, $P < 0.05$) but not in test two (see figure 2). No other significant effects were observed. In test one, eleven out of twelve birds consumed more QFR food reward than AL food reward. The remaining birds consumed more AL food reward than QFR food reward in test one.

FIGURE 2 GOES HERE

3.3.4. Total quantity of food consumed in each test

There was a significant effect of diet fed on day of testing on combined (total) consumption of the food reward during testing ($F_{1,10} = 9.50$, $P = 0.012$), with hungry birds (QFR fed) consuming more (mean \pm S.E.M.) food reward during the five minute test period (44.3 ± 4.2 g) than satiated (AL fed) birds (35.0 ± 3.7 g).

3.3.5. Anecdotal observations

As in the pilot study, it was observed that the birds demonstrated exaggerated gaping behaviour and neck ripples during swallowing whilst consuming the food reward.

3.4. Discussion

The birds showed a SDL preference in test one but not in test two. This finding was problematic to interpret; however, the continued confounding variable of unequal intake of food rewards paired with the diet options was a bigger issue. Therefore, it was necessary to conduct a third experiment to try and equalise intake of food rewards during training before SDL testing was conducted.

4. SDL Experiment 3

4.1. Introduction

The aim of this experiment was to equalise intake of the food reward during training (by making the food rewards more palatable) in order to identify if a SDL would remain once birds had had equal dietary exposure to each coloured food reward. The time taken to consume the food reward during the SDL tests was reduced to 3 minutes (as in experiment one) in case this had in some way influenced lack of SDL preference in test two (e.g. binge eating has been associated with a subsequent aversion to the food binged upon, Hertel and Eikelboom, 2010).

4.2. Materials and methods

4.2.1. Subjects

This study used 8 Ross 308 broiler breeders obtained as day old chicks from a commercial hatchery (Aviagen, Stratford – upon – Avon, UK). They were group reared and spot – brooded according to producer recommendations for heating and temperature (Aviagen 2006). The mean (\pm S.E.M.) bodyweight of the birds was 500 (\pm 30) g on day 29 (start of alternating between daily diet options) and 2319 (\pm 24) g on day 67 (end of study).

4.2.2. Housing and husbandry

From day 29 birds were individually housed in mesh cages (0.6m L \times 0.6m W \times 0.8m H) in blocks of four cages (two cages back – to – back) with each adjacent cage housing a conspecific. The cage floor was covered with a wooden tray filled with shavings to facilitate some natural behaviour. Water was available *ad libitum* and each bird was fed from a D – cup feeder located at the outermost corner of the cage. This was done to ensure that, whilst the other birds could see that a bird was feeding, they could not see what was being consumed, as this was essential to the study once the coloured food rewards were introduced. Facility housing and ethical / legal considerations (this final experiment was carried out at a different establishment and not under Home Office regulation) prevented the birds from being further separated either temporally or visually. Birds were weighed weekly throughout the experimental phase.

4.2.3. Food and nutrition

All birds were fed at 09:00h and alternated every two days between QFR and AL (*ad libitum* access to food between 09:00h and 17:00h (days 29 – 48) or 09:00-14:30h (day 49 onwards). Any food remaining at 17:00h (14:30h from day 49) was removed, weighed and intake recorded.

The basal diets consumed by all the birds was designed to meet the nutritional requirements of broiler breeders during the starter (day 1 – 35) and grower (day 36 onwards) phases (Target Feeds, Shropshire, UK). The starter mash contained 221 g CP / kg and 12.6 MJ ME / kg and the grower mash contained 211 g CP / kg and 13.2 MJ ME / kg.

The food rewards used in the SDL phase were 15 g of basal ration stained either red or green using food colouring (Silverspoon, Cambridgeshire, UK) in a mix of one part food colouring to two parts water. To each 15 g portion of the basal diet was added 2.25 ml of the mixture. Diets were fed moist and not dried in a warm oven prior to use (unlike in the previous experiments) as water is reported to improve diet palatability (Moritz et al., 2001).

4.2.4. SDL protocol

SDL training and testing started on day 49 and finished on day 68. The SDL protocol was similar to the previous two experiments with the following exceptions. On SDL training days any food remaining was removed at 14:30h and replaced with a food reward. On the two test days, all birds were tested between 14:30 – 16:30h.

Each SDL test lasted for three minutes. The birds were offered 70 g each of the red and green food rewards and the test and data collection was conducted as previously described for experiments one and two.

4.2.5. Statistical analysis

All statistical analyses were carried out using a repeated measures REML as detailed in experiment two.

4.3. Results

4.3.1. Daily food consumption during SDL training and testing

All birds always consumed the full ration of QFR on all of the days that it was offered. To allow for growth over the course of the experiment, the quantity offered was increased in 2 g increments over

the period of training and testing (from 43 – 49 g/day). The birds consumed considerably more feed on days that they were fed *ad libitum*. The mean daily quantity (\pm S.E.M.) consumed on AL days over the duration of training and testing was 156.2 (\pm 5.4) g.

4.3.2. Food reward consumption on days of SDL training

In contrast to experiments one and two, all birds consumed the full food reward offered on each day of SDL training (irrespective of either treatment or diet option fed that day).

4.3.3. SDL preference test

The initial analysis indicated that food reward consumption was affected by either test or food reward type ($F_{3,2} = 25.55$, $P = 0.038$). No other effects were significant. Post – hoc analysis using least significant differences at the 5% level indicated that birds consumed more of the QFR associated food reward than the AL associated food reward during test one (L.S.D = 13.73, $P < 0.05$) but not in test two (see figure 3). No other significant effects were observed. In test one, six out of eight birds consumed more QFR food reward than AL food reward. The remaining two birds consumed more AL food reward than QFR food reward in test one.

FIGURE 3 GOES HERE

4.3.4. Total quantity of food consumed during each test

The mean (S.E.M.) quantity of food reward consumed during testing was 22.4 (\pm 3.3)g when tested on the day the bird was fed QFR and 16.3 (\pm 2.5)g on the day the bird was fed AL. However, this was not statistically significant ($F_{1,7} = 1.34$, $P = 0.285$).

4.3.5. Anecdotal observations

Unlike in the previous two experiments the birds did not show any unexpected feeding behaviours such as exaggerated gaping or neck ripples.

5. Overall discussion

5.1. Study findings

The lack of SDL preference observed in test two of experiment two and three (but observed in test one) indicated that SDL preferences are not as robust a phenomenon as previously considered. Further, differential food reward consumption during training of the food rewards associated with AL and QFR were not essential to the development of SDL preferences as SDL preferences were still

observed when intake was equalised during training. Campbell et al. (1987) found a SDL preference (albeit for the low deprivation reward) in rats in which intake of rewards was matched during training under different states of food restriction. Whereas, SDL preferences towards the solution that was consumed in a lower quantity during training have also been observed (Capaldi et al., 1983). This indicates that quantity consumed during training may not necessarily be the cause of any preference observed during testing.

The actual reward may affect the SDL preference as when intake during training is matched, rats develop a SDL preference for sucrose but not saccharin solutions (Capaldi et al., 1994). This suggests that where nutritious food rewards (such as the coloured starter crumb or mash used in the current studies) are used, a SDL preference may develop regardless of whether food reward intake is equal or unequal during training. The composition of the food reward has been found to affect rat preferences for a food reward associated with high deprivation. Rats showed an SDL preference when the food reward was unsweetened mash but no preference when the mash contained sweeteners (Capaldi et al., 1991). Our study found that an SDL preference developed across two types of food reward (dry and moist). However, the nutritional profile of the food reward was similar and the food dyes identical across all experiments in our study.

The behaviour of the birds during testing in experiments one and two (exaggerated gaping and odd 'neck ripples' during swallowing) suggested that consuming the food was not pleasant (although an alternative explanation could be the dryness of the food made the food harder to swallow). These were not observed when feeding the moist food reward in experiment three suggesting that birds developed an SDL regardless of food reward palatability. However, this requires further investigation as other dimensions differed between diets as well. Campbell et al. (1987) performed a series of experiments aimed at identifying whether food reward linked SDL preferences formed due to an aversion for the less preferred reward or an increased attraction to the preferred reward. They concluded that the conditioning phenomenon was caused, not by increased aversion to one of the flavour rewards, but by increased attraction to the other flavour reward. Although, it should be noted, that Campbell et al. (1987) did not use food rewards that were inherently unpleasant to consume whereas the behaviour of the birds in experiments one and two suggested that the food rewards were not particularly liked.

The findings related to the total food reward intake during the test deserve further consideration. A significant difference in total food reward intake in birds tested on days when they have been fed QFR compared to days when they were fed AL was observed only in the second experiment. One interpretation would be that, despite the large difference in quantity of food consumed under each diet option, the birds were not sufficiently different in their hunger state at the point of testing in either experiment one and / or experiment three. However, in all three experiments, the mean intake of food reward was numerically lower on days when the bird was tested on a day when it was fed AL. Therefore, it is more reasonable to assume that the three minutes test period was simply not long enough for state – driven differences in intake to be observed even in older birds in which the contrast in feed intake between QFR and AL was more severe. Further, reducing the length of the test (from five minutes to three minutes) did not result in robust evidence of SDL derived preference being maintained into test two. Therefore, future studies should consider maintaining a five minute test period in order to maintain additional evidence of differences in hunger state between birds tested on QFR and AL days. This ensures validation of differential hunger state and motivational drive to consume food at the time of testing.

5.2. Methodological issues

A number of issues are relevant to any discussion of methodology. These include sample size, number of trials, the use of coloured food rewards and the use of cues which were concurrent with the reward itself. The sample sizes (per treatment group) used in the current study were four (experiment one), twelve (experiment two) and eight (experiment three). Although experiment one was small (it was intended as a pilot), the sample sizes used in experiments two and three are similar to sample sizes that yielded significant results for other authors (e.g. $n = 4$, Vasconcelos and Urcuioli, 2008; $n = 6$, Pompilio and Kacelnik, 2005; $n = 8$, Clement et al., 2000; $n = 12$, Kacelnik and Marsh, 2002; Friedrich and Zentall, 2004; Marsh et al., 2004) although less than others (e.g. $n = 13$, Aw et al., 2009; $n = 16$, Gipson et al., 2009). The number of stimulus / food reward – deprivation state pairings during training was four per state prior to the first test and eight by the second test in the our study. This is much less than the number of trials that it took for animals to learn to associate a distal cue with a food reward under two different states (e.g. 40 trials, Marsh et al., 2004; 120 trials, Vasconcelos and

Urcuioli, 2008). However, using a methodology in which the flavour of the food was the cue, Capaldi et al. (1983) observed a SDL after three flavour – deprivation pairings. This suggests that tasks where the cue is part of the food reward (such as a flavour) are easier to learn than cues that are more distal to the food reward. Despite this, Zentall (2008) suggests that, where only a few training trials are conducted, SDL researchers are less likely to observe an SDL or to find that any association between state and reward is more easily reversed. Where more than one SDL preference test is carried out on the same animal, very few of the published papers on SDL preferences report whether any order effects were either tested for or identified. Where order effects were investigated, evidence was mixed with some studies reporting no effect (Aw et al., 2009; Capaldi, et al., 1991; but see: Capaldi, et al., 1983).

The current experiments used identical food rewards stained different colours with food colouring. Chickens have good colour vision, and various studies have made use of their ability to recognise differently coloured foods (including contrasts between green and red substrates, e.g. Hothersall, et al., 2012; Rowe and Skelhorn 2005; Roper and Marples, 1997). Although chicks show innate preferences (or aversions) for food stained different colours (Roper and Marples, 1997), these preferences are modified where the outcome of feed consumption is rewarded (Kutlu and Forbes, 1993). No significant preferences for colour or interactions with other effects were observed in this study suggesting that colour biases were not a problem.

Coloured food rewards were used to more closely link the stimulus that cued food reward with the act of consuming the food. Previous studies by the authors suggested that hungry broiler breeders found distal cues (e.g. Y – maze colour arms) to signify differences in food quality or quantity difficult to learn (Buckley et al., 2011a, 2011b). Further, maze methods such as those utilised by Aw et al. (2009), Pompilio et al. (2006) and Kurtz and Jarka (1968) would be problematic to interpret as deprivation state has been shown to enhance side biases (Talling et al., 2002) and was a serious impediment in previous studies by the authors (Buckley et al., 2011a, unpublished observations). Thus, the experiments in this paper aimed at the opposite approach: coupling stimulus with reward to maximise associative strength. However, this may have affected SDL development. Most of the previous studies that observed a SDL preference for the stimulus associated with high deprivation

used cues distal to the food reward. In other words, the cues used were linked in some way to appetitive behaviour rather than consummatory behaviour. For example, pecking keys (Gipson et al., 2009; Pompilio and Kacelnik, 2005; Friedrich and Zentall, 2004; Marsh et al., 2004), positional cues (Aw et al., 2009; Kurtz and Jarka, 1968), colour cues (maze arms; Aw et al., 2009) or distance flown (Kacelnik and Marsh, 2002). SDL preferences for the reward associated with high deprivation have been identified when the cue is more closely linked with consummatory behaviour: by intrinsic characteristics of the food reward (e.g. scent, Pompilio et al., 2006; flavour, Capaldi et al., 1994; Capaldi et al., 1991). However, the opposite effect has also been observed with animals showing a preference for the reward associated with low deprivation (e.g. flavour, Capaldi et al., 1983; Capaldi and Myers, 1982) or no effect at all (e.g. flavour and artificial sweeteners, Capaldi et al., 1994). This suggests that the phenomenon of SDL is neither as robust, nor the predicted direction of effect as clear cut, when using rewards in which the conditioned stimulus associated with state of deprivation is the actual reward consumed.

The timing of the food rewards (closeness in proximity to daily feeding; Capaldi and Myers, 1982) has been shown to affect the direction of the SDL preference. Food rewards given just before and after feeding result in conditioned preferences for the high deprivation food reward (Revuksy, 1967). By contrast, food rewards given at a longer time interval from the start and finish of daily food consumption condition a preference for the low deprivation food reward (Capaldi et al., 1983; Capaldi and Myers, 1982) although not always (Capaldi et al., 1991). In our experiments the low deprivation reward was given directly after AL. However, the high deprivation food reward was 6 – 7 hours after the last daily meal and was eaten 15 – 18 hours before the next daily meal. This may have affected the development of SDL preferences. Further, both the presentation and the quantity of the food reward demonstrably affect SDL preference presence and direction (Capaldi et al., 1991). Rats given unsweetened mash show a preference for the reward associated with high deprivation regardless of quantity of reward offered (1 g or 16 g per training session). However, sweetening the mash with saccharin inhibited SDL preferences when the food reward was 16 g but not 1 g and increasing this artificial sweetness further inhibited SDL preferences with the smaller reward also. Further research is needed to quantify the effect of deprivation state on preference for different macronutrients and

energy density of food rewards to further understand the effect of deprivation state on SDL preference development (Capaldi et al., 1991) as these are likely to interact to influence both the development and direction of any SDL preference.

6. Conclusions and recommendations for future research

In conclusion, there was limited evidence of an SDL preference regardless of whether food reward intake during training was matched or different between hunger states and this deserves further exploration. However, the literature suggests both the development and direction of SDL preferences is complex, nutrient and context specific when using a methodology in which the cue linked to deprivation state is also identical with the food reward. This additional complexity may limit the value of SDL methodologies similar to the one used here. Further research should concentrate on using distal, appetitive cues, and include more training trials prior to testing.

7. Acknowledgements

The Universities Federation for Animal Welfare (UFAW) supported this research through a PhD studentship for Louise Buckley. SRUC is supported by the Rural and Environment Research and Analysis Directorate of the Scottish Government. The Roslin Institute is supported by an Institute Strategic Programme Grant from the BBRSC. Thanks are also due to Aviagen (Midlothian, Scotland) for donating the broiler breeder chicks used in experiment three. Becky Hothersall is also acknowledged for her useful support in relation to colour vision in poultry. None of the supporting organisations named had any further involvement in this study.

8. References

- Aviagen. 2006. Ross 308: Parent stock management manual. Aviagen, Midlothian, UK. pp. 1 – 86
- Aviagen. 2007. Ross 308: Parent stock performance objectives. Aviagen. Midlothian, UK. pp. 1 – 19
- Aw, J. M., Holbrook, R. I., Burt de Perera, T. and Kacelnik, A. 2009. State – dependent valuation learning in fish: banded tetras prefer stimuli associated with greater past deprivation. *Behav. Processes* 81, 333 – 336

536 Buckley, L. A., Sandilands, V., Tolkamp, B. J. and D'Eath, R. B. 2011a. Quantifying hungry broiler
537 breeder preferences using a closed economy T – maze task. *Appl. Anim. Behav. Sci.* 133, 216 – 227

538 Buckley, L. A., McMillan, L. M., Sandilands, V., Tolkamp, B. J., Hocking, P. M. and D'Eath, R. B.
539 2011b. Too hungry to learn? Hungry broiler breeders fail to learn a Y – maze food quantity
540 discrimination task. *Anim. Welf.* 20, 469 – 481

541 Buckley, L. A., Sandilands, V., Hocking, P. M., Tolkamp, B. J. and D'Eath, R. B. 2012. The use of
542 conditioned place preference to determine broiler preferences for quantitative or qualitative dietary
543 restriction. *Brit. Poult. Sci.* 53, 291 – 306

544 Campbell, D. H., Capaldi, E. D. and Myers, D. 1987. Conditioned flavour preferences as a function of
545 deprivation level: preferences or aversions? *Anim. Learn. Behav.* 15, 193 – 200

546 Capaldi, E. D. and Myers, D. E. 1982. Taste preferences as a function of food deprivation during
547 original taste exposure. *Anim. Learn. Behav.* 10, 211 - 219

548 Capaldi, E. D., Myers, D. E., Campbell, D. H. and Sheffer, J. D. 1983. Conditioned flavor preferences
549 based on hunger level during original flavour exposure. *Anim. Learn. Behav.* 11, 107 – 115

550 Capaldi, E. D., Sheffer, J. and Owebs, J. 1991. Food deprivation and conditioned flavour preferences
551 based on sweetened and unsweetened foods. *Anim. Learn. Behav.* 19, 361 - 368

552 Capaldi, E. D., Owens, J. and Palmer, K. A. 1994. Effects of food deprivation on learning and
553 expression of flavour preferences conditioned by saccharin or sucrose. *Anim. Learn. Behav.* 22, 173 –
554 180

555 Clement, T. S., Feltus, J. R., Kaiser, D. H. and Zentall, T. R. 2000. 'Work ethic' in pigeons: reward is
556 directly related to the effort or time required to obtain the reward. *Psychon. B. Rev.* 7, 100 – 106

557 D'Eath, R. B., Tolkamp, B. J., Kyriazakis, I. and Lawrence, A. B. 2009. 'Freedom from hunger' and
558 preventing obesity: the animal welfare implications of reducing food quantity or quality. *Anim.*
559 *Behav.* 77, 275 – 288

560 De Jong, I. C., van Voorst, A. S. and Blokhuis, H. J. 2003. Parameters for the quantification of hunger
561 in broilers. *Poult. Sci.* 84, 194 – 203

562 Friedrich, A. M. and Zentall, T. R. 2004. Pigeons shift their preference towards locations of food that
563 take more effort to obtain. *Behav. Processes* 67, 405 – 415

564 Gipson, C. D., Miller, H. C., Alessandri, J. J. D. and Zentall, T. R. 2009. Within trial contrast: the
 565 effect of probability of reinforcement in training. *Behav. Processes* 82, 126 – 132
 566 Hertel, A. and Eikelboom, R. 2010. Can overeating condition taste avoidance in previously food
 567 restricted rats? *Physio. Behav.* 99, 482 – 486
 568 Hocking, P. M., Zaczek, V., Jones, E. K. M. and Macleod, M. G. 2004. Different concentrations and
 569 sources of dietary fibre may improve the welfare of female broiler breeders. *Brit. Poult. Sci.* 45, 9 –
 570 19
 571 Hothersall, B., Caplen, G., Rose, K., Nicol, C. and Knowles, T. 2012. Food colour preferences in
 572 immature red junglefowl and broiler chickens. 46th Congress of the International Society for Applied
 573 Ethology, July 31st – August 4th, Austria, Vienna
 574 Kacelnik, A and Marsh, B. 2002. Cost can increase preference in starlings. *Anim. Behav.* 63, 245 -
 575 250
 576 Kurtz, K. H. and Jarka, R. G. 1968. Position preference based on differential food privation. *J. Com.*
 577 *Physiol. Psych.* 66, 518 – 521
 578 Kutlu, H. R. and Forbes, J. M. 1993. Self – selection of ascorbic acid in coloured foods by heat
 579 stressed broiler chicks. *Physio. Behav.* 53, 103 - 110
 580 Marsh, B., Schuck-Paim, C. and Kacelnik, A. 2004. Energetic state during learning affects foraging
 581 choices in starlings. *Behav. Ecol.* 15, 396 – 399
 582 Moritz, J. S., Beyer, R. S., Wilson, K. J., Cramer, K. R., McKinney, L. J. and Fairchild, F. J. 2001.
 583 Effect of moisture addition at the mixer to a corn-soybean-based diet on broiler performance. *J. Appl.*
 584 *Poultry Res.* 10, 347 – 353
 585 Nielsen, B. L., Thodberg, K., Malmkvist, J. and Steenfeldt, S. 2011. Proportion of insoluble fibre in
 586 the diet affects behaviour and hunger in broiler breeders growing at similar rates. *Animal* 5 – 8, 1247
 587 – 1258
 588 Nielsen, B. L., Litherland, M. and Noddegaard, F. 2003. Effects of qualitative and quantitative feed
 589 restriction on the activity of broiler chickens. *Appl. Anim. Behav. Sci.* 83, 309 – 323
 590 Pompilio, L., Kacelnik, A., Spencer, T. Behmer 2006. State – dependent learned valuation drives
 591 choice in an invertebrate. *Science* 311, 1613 – 1615

592 Pompilio, L. and Kacelnik, A. 2005. State – dependent learning and suboptimal choice: when starlings
 593 prefer long over short delays to food. *Anim. Behav.* 70, 571 – 578
 594 Revusky, S. H. 1967. Hunger level during food consumption: effects on subsequent preference.
 595 *Psychon. Sci.* 7, 109 – 110
 596 Roper, T. J. and Marples, N. M. (1997) Colour preferences of domestic chicks in relation to food and
 597 water presentation. *Appl. Anim. Behav. Sci.* 54, 207 – 213
 598 Rowe, C. and Skelhorn, J. 2005. Colour biases are a question of taste. *Anim. Behav.* 69, 587 – 594
 599 Sandilands, V., Tolkamp, B. J. and Kyriazakis, I. (2005) Behaviour of food restricted broilers during
 600 rearing and lay – effects of an alternative feeding method. *Physio. Behav.* 85, 115 – 123
 601 Rozenboim, I., Kapkowska, E., Robinzon, B. and Uni, Z. (1999) Effects of fenfluramine on
 602 bodyweight, feed intake and reproductive activities of broiler breeder hens. *Poult. Sci.* 78, 1768 –
 603 1772
 604 Sandilands, V., Tolkamp, B. J., Savory, C. J. and Kyriazakis, I. (2006) Behaviour and welfare of
 605 broiler breeders fed qualitatively restricted diets during rearing: are there viable alternatives to
 606 quantitative restriction? *Appl. Anim. Behav. Sci.* 96, 53 – 67
 607 Savory, J. S. and Lariviere, J. M. (2000) Effects of qualitative and quantitative food restriction
 608 treatments on feeding motivational state and general activity level of growing broiler breeders. *Appl.*
 609 *Anim. Behav. Sci.* 69, 135 – 147
 610 Talling, J. C., Inglis, I. R., Van Driel, K. S., Young, J. and Giles, S. (2002) Effect of hunger on
 611 starlings' preferences for food sources associated with variability or uncertainty. *Behav.* 139, 1223 –
 612 1235
 613 Vasconcelos, M. and Urcuioli, P. J. (2008) Deprivation level and choice in pigeons: a test of within –
 614 trial contrast. *Learn. Behav.* 36, 12 – 18
 615 Zentall, T. 2. (2008), Within trial contrast: when you see it and when you don't. *Learn. Behav.* 36, 19
 616 – 22